Nutritive value in whole plant maize forage: development of a predictive model by near infrared reflectance spectroscopy

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Introduction

Maize is an important forage species in the European Union; more than 3.5 million hectares are harvested annually for use as whole plant silage for many dairy and beef operations, because of its high energy content and ingestibility.[1] In addition to resistance against biotic and abiotic stress, the main objectives for breeding forage maize are: (i) improvement of whole plant dry matter yield, (ii) enhancement of feeding quality for ruminants and (iii) suitable dry matter concentration of forage to ensure proper fermentation for intake by livestock.[1]

Increased nutritional demands for optimum animal performance challenge maize production to select and manage hybrids for high dry matter yield with favourable quality traits.[1] Studies in this field have shown that the nutritive value of maize used for silage is a function of protein content, digestibility and intake potential and varies with both grain content and stover composition.[2] Grain is highly digestible and, typically, amounts to 50% of total dry matter under good growing conditions.[3] However, correlations between grain yield and forage yield were not high enough to justify selection on the basis of grain yield alone.[4] In this context, significant genotypic variation for digestibility and yield of stover was frequently reported among maize strains.[2,5–8] In addition, the results of these works suggested that variation in dry matter digestibility was closely related to decreases in fibre concentration of the stover plant rather than to variation in ear/stover ratio. Moreover, significant genetic variation exists for acid detergent fibre (ADF), neutral detergent fibre (NDF), cell wall (CW) and crude protein (CP) content of maize.[10,11] Altogether, these results suggest that the non-grain portion of the maize plant offers considerable potential for the improvement in yield and quality of forage.

Among the selection criteria proposed for selecting superior forage maize hybrids, harvesting the whole plant to measure total dry matter yield and determining the metabolisable energy concentration by near infrared (NIR) reflectance spectroscopy is the most economic procedure for the simultaneous evolution of yield and quality-related traits. NIR reflectance spectroscopy is a rapid method for predicting forage quality[12] and to estimate fibre concentration and *in vitro* dry matter digestibility[13–16] of forage grasses. If NIR reflectance spectroscopy equations could be developed that predict organic matter digestibility accurately, maize breeders may evaluate much larger populations than is possible with standard *in vitro* techniques, thus reducing expenses and expediting the time for the development of superior maize forage hybrids. The purposes of this study were to : (i) relate the biochemical parameters with a prediction equation for chemical and biochemical parameters for the whole plant of maize hybrids grown in different Italian environments during the last four years; (ii) develop a predictive

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model for estimating the nutritive value obtained from chemical and biochemical parameters using NIR reflectance spectroscopy.

Materials and methods

Four sets of commercial maize hybrids, belonging to different FAO maturity classes, ranging from 400 to 700, were evaluated by conventional experimental protocols at eight locations in Northern Italy between 1995 and 1998. Biomass yield and the dry matter content of the whole plant were measured in each experimental trial. A representative sample of 1–1.5 kg chopped material per plot was taken for chemical analyses, dry matter content and enzymatic digestibility measurements. Samples were dried at 65°C in a forced air oven to constant weight. After cooling and weighing, the samples were ground to pass a 1 mm screen. The samples were subsequently re-ground in a Cyclotec mill (Tecator, Hoganas, Sweden) through a 1 mm sieve. Before NIR reflectance spectroscopy analysis, samples were dried overnight at 40°C. Absorbance spectra ($\log 1/R$) of all samples were recorded in duplicate, using a Foss Electric Model 6500 scanning monochromator with a range 400–2500 nm. Two different sets of samples, with a wide range of chemical composition, representing hybrids of different class of maturity, years and locations, were used in this study; a set containing 673 samples, chosen by the SELECT algorithm,[17] was used to calibrate and to cross-validate the prediction equations. The other set of samples (from 129 to 156) was used for testing the goodness-of-fit of the developed prediction equations. Organic matter (OM) content was determined by ashing at 550°C for 6 h; in addition, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were carried out according to standard protocol reported in AOAC methods (1980). All chemical analyses were replicated twice. *In vitro* organic matter digestibility(OMD) was estimated according to Aufrere *et al.*,[18] while nutritive value expressed as milk feed unit per kg of dry matter (MFU) was computed according to the Andrieu and Demarquilly model.[19] Table 1 gives a summary of the chemical composition of samples used in the calibration. Near infrared spectroscopic calibration equations were developed for OM, CP, NDF, ADF, ADL, *in vitro* OMD and MFU. Equations for NIR reflectance spectroscopy prediction were developed using the Infrasoft International (ISI, Port Matilda, PA, USA) NIRS 4.2 software program "CALIBRATE" with the modified partial least squares regression option

Table 1. Mean values, ranges of variation and standard errors (*SE*) of chemical parameters and nutritive value used in the calibration set for maize forage samples.[a]

Quality related traits	*n* samples	Mean	Min.	Max.	*SE*
Organic matter	673	95.54	88.55	98.78	1.56
Crude protein	673	8.18	4.02	11.19	1.18
NDF	673	49.95	12.81	83.13	12.87
ADF	673	26.82	5.40	49.99	8.80
ADL	673	2.37	0.19	8.89	1.13
CF	673	21.45	4.32	39.99	7.04
OMD	673	62.98	32.72	91.94	11.80
Milk Feed Unit	673	0.79	0.32	1.35	0.20

[a]Chemical parameters are expressed in percentage (%) on dry matter basis, OMD in % of organic matter and MFU in kg dry matter

after elimination of outliers.[20] The math treatments 1, 4, 4 and 2, 10, 10 (first or second derivative, gap over which derivative was calculated, number of data points used in first smoothing and no second smoothing) were used for all prediction equations.

The following models were tested for chemical composition, enzymatic digestibility and NIR reflectance spectroscopy analysis: (i) OMD_1 = ADF; (ii) OMD_2 = ADF, NDF; (iii) OMD_3 = ADF, NDF, ADL; (iv) OMD_4 = NIRS MPLS (on reflectance and derivative function); (v) MFU_1 = OMD; (vi) MFU_2 = OMD, NDF; (vii) MFU_3 = OMD, ADF, NDF; (viii) MFU_4 = NIRS MPLS (on reflectance and derivative function).

Results and discussion

The overall means, their ranges of variation and the standard errors (*SE*) for the forage quality-related traits in the samples used in the calibration set are summarised in Table 1. In the set sample of 673, the difference in variation was appreciably large for most of the chemical parameters measured and for nutritive value, with the exception of organic matter (OM) for which a narrow range of variation was detected. These findings indicated that the samples examined here covered most of the variation frequently reported in the literature for whole plant maize forage.[8,16,21] In fact, the mean of crude protein (CP) concentration ranged from 4.02% to 11.19%, while the percentage of the cell wall and structural components, expressed as NDF, ADF and ADL, ranged, respectively, from 12.81% to 83.13%, from 5.40% to 49.99% and from 0.19% to 8.89%. Also, the nutritive value indexes measured as a percentage of OM, the *in vitro* organic matter digestibility coefficient (OMD) and the milk feed unit (MFU), showed a sizeable variation, ranging, respectively, from 32.72% to 91.94% and from 0.32 units to 1.35 units, with the exception of organic matter in which a narrow range of variations was noted (88.55% to 98.78%).

Statistics are reported in Table 2 , including standard errors of calibration (*SEC*) and R^2 values for the equations of best fit obtained for each forage quality-related traits herein studied, the number of modified partial least square (MPLS) terms and the number of wavelengths (λ) used in developing the calibration equations. In the same table, the r^2 values for the cross-validation and standard error of cross validation (*SECV*) are also reported. The standard errors of calibration were of acceptable quality ($R^2 > 0.80$) for all traits examined, except for ADL, in which a lower coefficient of determination was found (0.75). Similarly, the same trends of response were obtained for all traits in the

Table 2. Statistics of the calibration equation of best fit and cross-validation, including the number of MPLS (modified partial least squares) factors and the number of wavelengths ( ) used in each equation, standard error of calibration (*SEC*) and standard error of cross-validation (*SECV*).

Quality related-traits	MPLS	R^2	*SEC*	r^2	*SECV*	λ
Organic Matter	15	0.94	0.33	0.92	0.37	168
Crude Protein	10	0.88	0.39	0.87	0.41	168
NDF	5	0.96	2.54	0.95	2.58	168
ADF	5	0.97	1.49	0.96	1.54	168
ADL	10	0.75	0.47	0.73	0.49	173
OMD	6	0.95	2.25	0.95	2.33	168
Milk Feed Unit	9	0.96	0.04	0.95	0.04	173

Table 3. Prediction mean values obtained on a separate sample set of maize hybrids[a]

Quality related-traits	Sample *n*	r^2	Laboratory means	*SE*	Predicted means	*SEP*
Organic Matter	156	0.94	89.10	3.19	90.92	0.47
Crude Protein	150	0.85	7.25	1.20	7.60	0.62
NDF	156	0.96	66.65	11.67	68.12	2.55
ADF	156	0.98	38.93	9.86	42.01	1.26
ADL	129	0.61	3.37	1.37	3.83	0.94
OMD	152	0.96	46.85	12.50	49.39	2.68
Milk Feed Unit	152	0.93	0.44	0.23	0.43	0.06

[a] r^2 is the coefficient of simple correlation, (*SE*) standard error of laboratory analyses and (*SEP*) standard error of prediction.

cross-validation procedure where r^2 values ranged from 0.73 for ADL to 0.96 for ADF; for all traits the standard errors of cross-validation were substantially low.

The precision of the estimates of the quality-related traits and nutritive value of whole-plant forage, as predicted by NIR reflectance spectroscopy, are shown in Table 3. The relationships between laboratory and NIR reflectance spectroscopy predicted parameters (r^2) were high for all traits ($r^2 > 0.90$), with the exception of crude protein (0.85) and, in particular, for ADL, where a lower correlation was noted (0.61); this is also confirmed by the low standard error of prediction (*SEP*) ranging from 0.06 to 2.55 of MFU and NDF, respectively. These results are in good agreement with those reported for the calibration set and are consistent with similar results reported earlier.[21,22] The *SEP* generally reflects the accuracy of the chemical determinations and the results shown in Table 3 are in good agreement with those obtained by conventional chemical analyses.

In Table 4, the models developed for OMD and MFU using chemical parameters and NIR reflectance spectroscopy spectra, are reported. The first three models for both parameters were obtained using a regression stepwise method, while for the fourth model a modified partial least squares method was adopted. The coefficients of multiple regression for organic matter digestibility were high, ranging from 0.83 for the first model, when only one chemical parameter, the ADF, was used, to 0.95 for the last model, when only spectra data detected by NIR reflectance spectroscopy was used. The standard error of estimates for OMD models varied from 4.82 for the first to 2.25 for the fourth model, indicating a two-fold increase in precision. For MFU models, the R^2 and *SEE* values were lower than those of OMD, ranging from 0.90 to 0.96 for R^2 and from 0.06 to 0.04 for *SEE*. Also, for MFU, the better estimation values were obtained using only spectral data detected by NIR reflectance spectroscopy instead of chemical or *in vitro* parameters.

The results of the comparison between the different methods for estimating chemical composition of maize forage and their ability to predict digestibility and MFU are comparable to those proposed by other authors.[16] In addition, our results are in accordance with previous observations reported by other authors[1,13] suggesting that NIR reflectance spectroscopy is a reliable technique in determining chemical composition of maize forage and is more accurate than enzymatic digestibility and chemical composition analyses in predicting the nutritive value of maize forage. Although these results show that NIR reflectance spectroscopy can be used as a tool to scan a large number of samples, nevertheless, the success of NIR reflectance spectroscopy analysis of forages depends almost entirely on the reliability of primary calibration data. When compared to conventional laboratory analyses, NIR reflectance

Table 4. Prediction models of *in vitro* organic matter digestibility (OMD in %) and milk feed unit (MFU) per kg of dry matter from chemical composition (% of dry matter) and absorbance (log 1/*R*).[a]

Equations					R^2	*SEE*
i	OMD_1 = 9.46	–0.87 ADF			0.83	4.82
ii	OMD_2 = 99.47	–0.55 ADF	–0.36 NDF		0.87	4.31
iii	OMD_3 = 98.98	–0.50 ADF	–0.34 NDF	–0.09 ADL	0.87	4.24
iv	OMD_4 = Absorbance	derivative	function		0.95	2.25
v	MFU_1 = –0.17	+ 0.91 OMD			0.90	0.06
vi	MFU_2 = 0.34	+ 0.65 OMD	–0.30 NDF	–0.30 NDF	0.93	0.05
vii	MFU_3 = 0.51	+ 0.54 OMD	–0.25 ADF	–0.15 NDF	0.94	0.05
viii	MFU_4 = Absorbance	derivative	function		0.96	0.04

[a] R^2 multiple regression coefficient, significant at ($P < 0.01$); *SEE* standard error of estimate except for model IV and VIII (standard error of cross-validation).

spectroscopy appears to be an attractive alternative technique to estimate forage related quality-traits, due to its speed, simplicity, safety and low operational costs. This is of particular importance in plant breeding programmes, where a large number of samples must be analysed in a short time to accelerate the development of superior quality forage maize hybrids.

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